

Screening of Factor V G1691A (Leiden) and Factor II/prothrombin G20210A Polymorphisms among Apparently Healthy Taif-Saudi Arabia Population Using a Reverse Hybridization Strip Assay Approach

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Abstract: Single nucleotide polymorphisms (SNPs) Factor V G1691A and Factor II G20210A have been shown to play a role in both venous and arterial thrombosis. Several studies have been carried out to estimate the prevalence of Factor V G1691A and the Factor II/prothrombin G20210A gene polymorphisms among healthy subjects. The aim of the present work is to study the prevalence and allele frequency of factor V G1691A (Leiden) and factor II/prothrombin G20210A polymorphisms among healthy Taif-Saudi Arabia population. The SNPs genotyping was carried out via Cardiovascular Disease (CVD) Strip Assay which is based on a Polymerase Chain Reaction-Reverse hybridization technique and DNA from 200 unrelated Saudi healthy subjects. The mutant (AA) genotype of both studied polymorphisms was completely absent. The prevalence of FV-Leiden and factor II/prothrombin G20210A were 2% and 1.5% respectively. This study identify the prevalence of Factor V G1691A and the Factor II/prothrombin G20210A polymorphisms which were generally comparable to the rates established for countries of Caucasian descent.

Key words: Factor V G1691A (Leiden) · Factor II/prothrombin G20210A · Polymorphisms · Saudi Arabia

INTRODUCTION

Cardiovascular disease (CVD) is the most common cause of death in developed and developing countries of the world. A number of candidate genes and genetic abnormalities may be involved in the pathophysiology of the CVD. Single nucleotide polymorphisms (SNPs) represent a part of risk factors that contribute to cardiovascular disease [1]. SNPs in the Factor V and Factor II or prothrombin have been shown to play a role in both venous and arterial thrombosis. In addition, it is now accepted that these SNPs are the most common inherited abnormalities in blood coagulation that lead to thrombophilia [2]. The gene encoding human Factor V is located on chromosome 1q21–25 [3]. The Factor V G1691A SNP, also referred to as the Factor V Leiden, results in the substitution of guanine by adenine at nucleotide 1691. This genetic change in turn results in

the replacement of glutamine for arginine at position 506. The resulting amino acid substitution due to this SNP slows down the proteolytic inactivation of Factor Va by activated protein C (activated protein C resistance), thereby increasing the generation of thrombin. This reduced anticoagulant effect of activated protein C leads to an increased tendency toward thrombosis [4].

The prothrombin gene consists of 14 exons spanning approximately 21 kb at position p11- q12 of chromosome 11 [5]. A single G to A polymorphism at nucleotide position 20210 was found to be associated with increased gene expression levels and can result in an increased risk for thrombophilia [6, 7].

Several studies have been carried out to estimate the prevalence of Factor V G1691A and the Factor II/prothrombin G20210A gene polymorphisms as well as study the association between these two mutations and different cardiovascular diseases [8-10].

Worldwide the prevalence of these two SNPs varies depending on the geographical location and the ethnic background of the population [4, 11].

Different studies have been conducted to estimate the prevalence of Factor V G1691A and the Factor II/prothrombin G20210A gene polymorphisms among Arabs [12-17]. Heterogeneity in the prevalence and allele frequencies of FV-Leiden and PRTG20210A was noted. This heterogeneity was described by very high prevalence of FV-Leiden in Lebanon [13, 18] and Jordan [19] and its virtual non existence in Morocco [20] and Algeria [21].

The aim of the present work was to report the prevalence of factor V G1691A (Leiden) and factor II/prothrombin G20210A polymorphisms among healthy Taif-Saudi Arabia population using a reverse hybridization strip assay approach.

MATERIALS AND METHODS

Samples Collection: Blood samples from 200 unrelated healthy Saudi subjects residing in Taif city with no cardiovascular disease symptoms were randomly collected into EDTA anticoagulant vacutainer tubes. Verbal consent was obtained from all participants prior to blood samples collection and all institutional requirements were met.

DNA Extraction and PCR and Reverse Hybridization: All studied samples were processed according to the CVD Strip Assay, Vienna Lab, Austria (<http://www.viennalab.com>) manufacture protocol.

The CVD Strip Assay is based on the in vitro reverse-hybridization principle and includes three successive steps: (1) DNA isolation (2) PCR amplification using biotinylated primers (3) hybridization of amplification products to a test strip containing allele specific oligonucleotide probes). Briefly, in a single multiplex polymerase reaction different gene sequences were amplified and biotin labeled. The thermal profile of PCR was as follow, initial step of 94°C for 2 min and followed by 35 cycles of 94°C for 15 s, 58°C for 30 s and 72°C for 30 s, final extension was at 72°C for 3 min. Test strips contains allele specific (wild type and mutation) oligonucleotide probes immobilized as an array of parallel lines. Bound biotinylated sequences are detected using streptavidin-alkaline phosphatase and color substrates. After series of stringent washes (according to the protocol of provider) the reaction was detected by color development directly on test strip. Results were evaluated from test strips using provided scale included in the kit.

RESULTS

Two hundred healthy Saudi individuals were included in the present work. In order to analyze the factor V G1691A (Leiden) and factor II/prothrombin G20210A polymorphisms, CVD Strip Assay was utilized. The banding patterns of CVD Strip Assay demonstrate the mutant and wild type for both of studied polymorphisms are shown in Fig. 1. For each individual one of three possible staining patterns may be obtained as follow; (1) Wild type probe only: Normal genotype (GG), (2) Wild type and mutant and mutant probe: Heterozygous

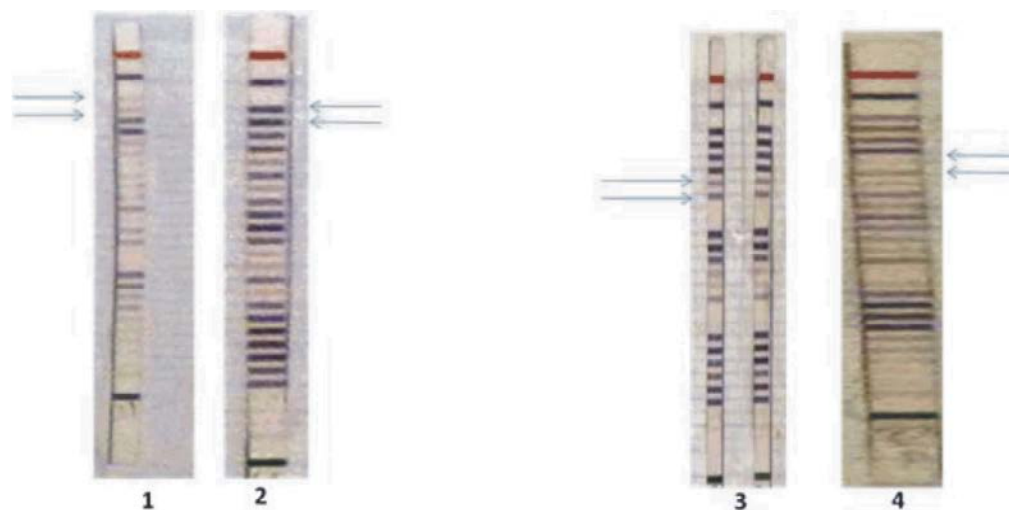


Fig. 1: Banding pattern of Test strips (CVD Strip Assay) demonstrate obtained Genotypes of Factor V G1691A (strips 1 and 2) and prothrombin G20210A (strips 3 and 4)

Table 1: Factor V G1691A (Leiden) and prothrombin G20210A Genotypes and alleles distributions

Polymorphism	Genotypes			Alleles	
	G/G	G/A	A/A	G	A
Factor V G1691A	196 (98%)	4 (2%)	0 (0.0)	0.99	0.01
Prothrombin G20210A	197 (98.5%)	3 (1.5%)	0 (0.0)	0.99	0.01

genotype (GA) and (3) Mutant type probe only: Homozygous mutant type (AA). The prevalence %, genotypes and allele frequencies for each studied polymorphism were tabulated in Table 1.

Homozygous mutant genotype (AA) for both two studied polymorphisms was absent among screened 200 healthy individuals. Two genotypes for factor V G1691A (Leiden) polymorphism were obtained. 196 individuals were normal genotype (G/G) with prevalence (98%) and four individuals proved to be a mutant as heterozygous genotype (GA) giving prevalence (2%).

The G and A alleles of factor V G1691A (Leiden) were detected with allelic frequencies 0.99 and 0.01 respectively. The observed genotypes of G20210A polymorphism were GG and GA with prevalence% (98.5) and (1.5) respectively. Whereas the allele frequency of A and G alleles was 0.01 and 0.99.

DISCUSSION

Based on the reported association between factor V G1691A (Leiden) and factor II/prothrombin G20210A polymorphisms and thrombophilia, various studied worldwide were conducted to identify the association between these mutations and different cardiovascular diseases [22-26]. Moreover different studies were carried out to estimate the prevalence and allele frequencies of these mutations among different healthy subjects in different countries [14, 15, 17].

In view of their role as inherited risk factor of venous thrombosis, coupled with their selective distribution in different world regions and ethnic-specific variation in allele frequency, we investigated the prevalence of V G1691A (Leiden) and factor II/prothrombin G20210A polymorphisms in Taif Saudi healthy population. The obtained results of Factor V G1691 A SNP reported that, mutant (AA) genotype was completely absent among the study subjects. The prevalence of FV-Leiden was 2%.

These results are in accordance to the findings of a previous study conducted among different ethnic groups including Africans, Koreans, Peruvian Indians, Chinese, Japanese, Mongolians, Taiwanese; Saudi and Bahraini [27-32].

The prevalence of FV-Leiden in Saudi Arabia was generally comparable to the rates established for countries of Caucasian descent. Given that the primary focus of the FV Leiden most likely lies in the Eastern Mediterranean basin [33, 34], it is plausible that the presence of FV- Leiden in the geographically distinct Saudi Arabia was brought about by the migration of mutation-carrying individuals and by the admixture of Eastern Mediterranean with Arabian Peninsula inhabitants, most notably during the Islamic expansion era [15].

The resulted genotypes of Factor II showed that, mutant genotype (AA) was completely absent. These findings are in agreement to the results of a previous study carried out among different ethnic groups including Whites, African, Brazilian Blacks, Asians, Amerindians and Lebanese [4, 11] as well as genetically isolated populations including the Inuit and Pima Indians [35, 36].

The prevalence of factor II/prothrombin G20210A in the present study was (1.5%). It is in comparable rates reported from European populations such as Irish, Netherlanders, English, Swedish, Austrians and Croatians, who reported an allelic frequency from 1 to 1.9% [37-41].

Study of the prevalence and allele frequency of these mutations among healthy individuals has a role in the set up a baseline data that may be important for future projects correlating these polymorphisms with variable clinical conditions [17]. Moreover, this information will be used in studies of gene-disease association, as well as for population genetics [15].

CONCLUSION

This study identifies the prevalence of FV-Leiden and factor II/prothrombin G20210A was 2% and 1.5% respectively. It is generally comparable to the rates established for countries of Caucasian descent. The mutant (AA) genotype of both Factor V G1691A and the Factor II/prothrombin G20210A polymorphisms was completely absent.

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